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TITLE: Polymerase enhancing factor (PEF) extracts PEF protein complexes isolated PEF proteins and methods for purifying and identifying same

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INVENTOR-INFORMATION:

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Hogrefe; Holly	San Diego	CA		

US-CL-CURRENT: 435/91.2; 536/23.7, 536/24.1

CLAIMS:

What is claimed is:

1. A non-naturally occurring composition of matter comprising at least one component possessing nucleic acid polymerase enhancing activity selected from the group consisting of: an isolated or purified naturally occurring polymerase enhancing protein obtained from an archeabacteria source; a wholly or partially synthetic protein having the same amino acid sequence as said naturally-occurring protein or analogs thereof possessing polymerase enhancing activity; polymerase-enhancing mixtures of one or more of said naturally occurring or wholly or partially synthetic proteins; polymerase-enhancing protein complexes of one or more of said naturally occurring or wholly or partially synthetic proteins; or polymerase-enhancing partially purified cell extracts containing one or more of said naturally occurring proteins.

2. A composition of matter according to claim 1 wherein said component possessing polymerase enhancing activity is a cell extract.

3. A composition of matter according to claim 2 wherein said cell extract is from an archeabacteria source.

4. A composition of matter according to claim 3 wherein said cell extract is from Pyrococcus furiosus.

5. A composition of matter according to claim 1 wherein said component possessing polymerase enhancing activity is a protein complex.

6. A composition of matter according to claim 5 wherein said protein complex is from an archeabacteria source.

7. A composition of matter according to claim 6 wherein said protein complex is from Pyrococcus furiosus.

8. A composition of matter according to claim 7 wherein said protein complex is P300.

9. A composition of matter according to claim 5 wherein said protein complex

comprises a plurality of subunits wherein at least one subunit has a molecular weight of approximately 45 kD.

10. A composition of matter according to claim 9 wherein a subunit has a sequence of amino acids at the amino terminal end comprising SEQ ID NO: 46.

11. A composition of matter according to claim 9 wherein a subunit has a sequence of amino acids comprising one of SEQ ID NO: 47 or 48.

12. A composition of matter according to claim 10 or 11 further comprising a subunit encoded by a DNA having the nucleotide sequence of SEQ ID NO: 18.

13. A composition of matter according to claim 1 wherein said component possessing polymerase enhancing activity is a protein.

14. A composition of matter according to claim 13 wherein said protein is from an archeabacteria source.

15. A composition of matter according to claim 14 wherein said protein is from Pyrococcus furiosus.

16. A composition of matter according to claim 15 wherein said protein comprises at least one protein having a molecular weight of approximately 45 kD.

17. A composition of matter according to claim 16 wherein said protein is selected from the group consisting of: a protein having a sequence of amino acids at the amino terminal end comprising one of SEQ ID NO: 11; a protein encoded by a nucleic acid having the sequence of SEQ ID NO: 42 or degenerate variants thereof; or a protein having a sequence of amino acids comprising one of SEQ ID NO: 37-39, 41, or 43-48.

18. A composition of matter according to claim 1 wherein said component possessing polymerase enhancing activity is a wholly or partially synthetic protein having the same amino acid sequence as said naturally-occurring protein or analogs thereof.

19. A composition of matter according to claim 18 wherein said protein has a molecular weight of approximately 45 kD.

20. A composition of matter according to claim 19 wherein said protein has a sequence of amino acids at the amino terminal end comprising SEQ ID NO: 3, 11 or 46.

21. A composition of matter according to claim 19 wherein said protein has a sequence of amino acids comprising one of SEQ ID NO: 5, 6, 47 or 48.

22. A composition of matter according to claim 20 or 21 further comprising a subunit encoded by a DNA having the nucleotide sequence of SEQ ID NO: 18.

23. A composition of matter according to claim 1 wherein said component possessing polymerase enhancing activity is a mixture of proteins.

24. An isolated or purified DNA comprising a sequence encoding a protein according to said protein of one of claims 16-17.

25. An isolated or purified DNA having a sequence selected from the group consisting of: the sequence set forth in SEQ ID NO: 18, degenerate sequences thereof, or DNA sequences hybridizable therewith; the sequence set forth in SEQ ID NO: 42, degenerate sequences thereof, or DNA sequences capable of hybridizing therewith.

26. An isolated or purified DNA sequence capable of hybridizing to DNA sequence according to claim 24.

27. An isolated or purified DNA sequence capable of hybridizing to DNA sequence

according to claim 20.

28. A composition of matter comprising a polymerase-enhancing protein encoded by DNA according to claim 26.

29. A composition of matter comprising a polymerase-enhancing protein encoded by DNA according to claim 27.

30. A non-naturally occurring mixture of a polymerase-enhancing composition according to claim 1 with one or more DNA polymerases.

31. A mixture according to claim 30 wherein at least one of said polymerases is a thermostable DNA polymerase.

32. A mixture according to claim 30 wherein at least one of said polymerases is derived from an archeabacteria source.

33. A mixture according to claim 32 wherein at least one of said polymerases is a DNA polymerase derived from the Pyrococcus species or the Thermococcus species.

34. A mixture according to claim 33 wherein at least one of said polymerases is Pyrococcus sp. JDF3, Pyrococcus sp. GBD, Pyrococcus sp. KOD, Thermococcus or Pyrococcus woesii DNA polymerase.

35. A kit for replicating nucleic acids comprising a polymerase-enhancing composition of claim 1 and at least one nucleic acid polymerase.

36. A kit according to claim 35 containing at least one recombinant nucleic acid polymerase.

37. A kit according to claim 35 or 36 capable of use in a site-directed mutagenesis method.

38. A kit according to claim 35 or 36 capable of use in a nucleic acid sequencing method.

39. A kit according to claim 35 or 36 capable of use in an amplification reaction.

40. An antibody that binds to a composition of matter of claim 1.

41. An antibody that binds to a protein having an amino acid sequence comprising one of SEQ ID NO: 19, 37-39, 41, 43-48.